REMARKS

Applicants have reviewed the file history of this case and carefully considered past actions by the Examiner and Applicants' attempts to advance this case by explanations distinguishing the claimed invention over documents cited by the Examiner as well as through various amendments to the claims made to provide clear claim language describing the process of the invention in a manner that is consistent with the written description of the invention found in the text and the many working examples of the specification. However, as prior amendments have not resulted in advancement of this application, Applicants have further amended Claims 1 and 51 to clearly state that the claimed process for isolating nucleic acids involves immobilizing nucleic acids to a non-siliceous membrane by *binding* the nucleic acids to one side of the non-siliceous membrane in the presence of an immobilization buffer (also referred to as binding solution) and subsequently releasing the immobilized nucleic acids from the non-siliceous membrane by applying an elution agent wherein the released nucleic acids do not pass through to the other side of the non-siliceous membrane. Support for the amendments is found in the specification, as indicated below:

> "Another aspect of this invention is, in particular, to *bind* nucleic acids to an immobile phase -- especially to a membrane . . . " (p. 3, lines 29-30, of the specification; emphasis added)
>
> "In any case, what is essential for the elution step, in the procedure on which this invention is based, is that the nucleic acids are removed from the same side of the membrane from which they were applied to the membrane, i.e., that there is *no passage of nucleic acids through the membrane*." (p. 11, lines 7-10, of the specification; emphasis added)
>
> "Under the conditions thus created, the RNA *remained bound* to the membrane." (Example 1, p. 16, lines 26-27, of the specification; emphasis added)
>
> "Under the conditions described, the RNA *remained bound* to the membranes. The membranes were then washed as described in Example 1." (Example 2, p. 18, lines 28-29, of the specification; emphasis added)

See, also, Tables 2-4 and 7-10, of the specification.

As noted previously in this record, various salts and alcohols that are useful in binding nucleic acids to one side of a non-siliceous membrane according to Applicants' invention are described throughout the specification (see, e.g., p. 6, line 29-p. 7, line 22, regarding salts; p. 7, line 23-p. 8, line 2, regarding useful alcohols, Examples 1-18; and note, also, studies of various binding solution components in Examples 8-13).

Likewise, elution agents used in the invention to release nucleic acids bound to a non-siliceous membrane are described in the specification (see, e.g., p. 9, line 26-p. 10, line 10 and Examples 1-19, of the specification).

The specification also makes clear that nucleic acids released from the non-siliceous membrane do not pass through to the other side of the non-siliceous membrane (see, e.g., p. 11, lines 7-10 and Examples 1-19, of the specification).

In view of the amendment to Claim 1 to specifically recite a non-siliceous *membrane*, Applicants have canceled Claim 32 to avoid double claiming and also amended Claims 33, 35, 36, and 39 to depend from Claim 1 instead of canceled Claim 32.

Within the context of independent Claims 1 and 51, as amended herein, Applicants have made other amendments to the dependent claims to refer to the expressed features of amended Claims 1 and 51 *and* to basically place the claims in a form similar to those of Applicants' Response of October 21, 2003. Such amendments include introducing dependent Claims 77-91. Support for new Claims 77-91 is found throughout the specification, including the original claims as indicated below:

New Claim	Original Claim
77	2
78	18
79	19
80	20
81	21
82	41
83	26, 44
84	27, 45
85	46
86	47
87	48
88	49
89	50, 58
90	41
91	41

The above amendments to the claims are thus made to recite features of the invention that would be evident to the skilled practitioner who reads Applicants' specification. Accordingly, the amendments add no new matter, and entry of the amendments is respectfully requested.

The amendments to the claims are also discussed further below along with the rejections made in the Office Action dated December 30, 2005.

Rejection Under 35 USC 112, first paragraph

In the Office Action, the Examiner rejected Claims 1, 3-5, 9-17, 19, 20, 24-40, 51, 53-57, 59-64, 69-74, and 76 under 35 USC 112, first paragraph, for failing to comply with the written description requirement. In particular, the Examiner objected to recitation of the term in Claims 1 and 51 of the phrase "in the absence of a cationic detergent" as lacking support from the specification. Without acceding to the Examiner's view in the Office Action, Applicants note that the claims, as amended above, do not recite the objected to phrase thereby rendering the Examiner's rejections moot.

Rejections Under 35 USC 103

The Examiner rejected Claims 1, 3-5, 9-17, 19, 20, 24-40, 51, 53-57, 59-64, 69-74, and 76 under 35 USC 103 as obvious over EP 0 431 905 ("Ogawa") in view of Pfister et al., *J. Biol. Chem., 271(3)*: 1687-1694 (1996) (as a reference for the RNeasy® product and process) and US 5,234,809 ("Boom"). Applicants respectfully traverse the rejections for the reasons provided below.

Regarding Ogawa, Pfister, and Boom, the Examiner stated:

> "Ogawa teaches a process for isolating DNA comprising applying a solution (triptone, NaCl and yeast extract) containing DNA and proteinase K to a membrane, *which can be any commercially available membrane*, for example polysulfone (non-siliceous). (e.g., col. 3, ll. 37-41). Ogawa teaches that washing with an appropriate buffer solution would increase yield, and gives TE-buffer as an example. (e.g., col. 3, ll. 45-50). Ogawa also teaches that DNA is released from the membrane using shaking in a volume of TE buffer, where the eluate is recovered by pipette (without penetration through the membrane or [contact] with opposing surface). (e.g., col. 4, ll. 35-39) [emphasis added]

"Ogawa does not explicitly teach the step of immobilizing nucleic acids to the non-siliceous membrane in the *presence of a salt and an alcohol*. However, the reference need not teach what is routinely practiced in the prior art. Indeed, utilizing salts such as guanidium isothiocyanate, as well as alcohol such as ethanol, was routinely practiced in the prior art in immobilizing nucleic acids (e.g., RNA) onto surfaces. [emphasis in original]

"For example, Pfister teaches that in order to purify nucleic acids (i.e., RNA) from culture the RNeasy® kit is utilized. The RNeasy® handbook teaches several protocols for isolating RNA from cell lysis, including adding buffer RLT (containing salt - guanidium isothiocyanate; as in the instant Specification, p. 19, Example 13) and adding ethanol to clarified lysis, which mixture is subsequently added to RNeasy membrane. [citation omitted] If it were contended that the Handbook is not analogous because the membrane utilized therein is siliceous, it is important to note that in methods of purifying nucleic acids, siliceous and non-siliceous membranes are often disclosed as supporting the same process - purifying nucleic acids - with the understanding that one of skill in the art will recognize that it would require nothing more than routine experimentation to optimize conditions relative to buffers or membranes.

"For example, Boom teaches methods of purifying nucleic acids using various buffers (columns 6-7). Further Boom teaches that the methods are suitable for various nucleic acids (e.g., RNA, dsDNA, ssDNA). (col. 8, ll. 15)

"However, most importantly, the reference teaches that various surfaces, including siliceous and non-siliceous (e.g., silica derivatives, latex, PVDF, nitrocellulose, Hybond-N), can be utilized in purifying nucleic acids. (e.g., col. 6, ll. 5-27) Thus the evidence in the art suggests that optimizing buffers and immobilizing surfaces constitutes nothing more than routine experimentation. As a result, Applicant's implicit assertion that employing a step of immobilizing nucleic acids to a non-siliceous surface *in the presence of a salt and an alcohol*, cannot be deemed nonobvious. [emphasis in original]

"As such, with the foregoing teachings and evidence, it would have been *prima facie* obvious to optimize the buffer conditions of Ogawa, so as to included salts and an alcohol, in practicing a method of purifying nucleic acids. One would have been motivated to optimize the buffer/membrane combinations,

> depending on the species of nucleic acids sought to be purified, given the broad limitation of "nucleic acids" to which the base claims are directed. Further, given the level of skill in the art at the time of the invention, there would have been a reasonable expectation of success in conducting routine experimentation to obtain optimum conditions for purifying a particular species of nucleic acids." (pp. 6-7, of the Office Action)

First, Applicants are well aware of the method of concentrating nucleic acids from a solution by alcohol precipitation of the nucleic acids in the presence of certain concentrations of salts. Applicants do not know whether some degree of alcohol precipitation of nucleic acids may take place under some conditions within the practice of Applicants' claimed process. What is clear is that in practicing Applicants' claimed process, nucleic acids are bound to one side of a non-siliceous membrane under one condition (in the presence of an immobilization buffer) and can then be released from the membrane under a different condition (in the presence of an elution agent) and retrieved from that same side of the membrane without passing through to the other side of the membrane. Applicants also do not contend that the various reagents employed in the claimed process are novel substances. Applicants' inventive method must be viewed as a whole, i.e., as presented and defined in the amended claims, not analyzed according to its individual parts.

Applicants note that Ogawa expressly teaches use of the process of ultrafiltration *instead* of various conventional methods such as ethanol precipitation of DNA and :

> "The widely adopted conventional method for purifying phage DNAs from the culture medium comprises the steps of removing *E. coli* cells from the culture medium by centrifugation, precipitating the phage by treatment with polyethyleneglycol, removing proteins by phenol extraction and *concentrating DNA by ethanol precipitation.*
>
> "However, with *this conventional method*, reagents which are toxic to human, such as phenol and chloroform must be used. Further, since centrifugation is necessary in each step, it is difficult to automatize the method. For automatizing the purification, a method which does not require centrifugation is desired.
>
> "SUMMARY OF THE INVENTION
> Accordingly, the object of the present invention is to provide a process of purifying phage DNAs by which phage DNAs may be

> purified to high purity without centrifugation steps and without using reagents toxic to human.
>
> "The present inventors intensively studied to find that phage DNAs may be purified to high purity by removing *E. coli* cells by filtration through a membrane filter, decomposing and denaturing the phage proteins, and then *removing the proteins by ultrafiltration, to complete the present invention.* [emphasis added] (col. 1, lines 17-24, 35-45, of Ogawa)

The above excerpt makes clear that use of an ***ultrafilter*** (ultrafiltration membrane) is the essence of Ogawa's invention as the ultrafiltration step "completes" the invention. Thus, contrary to the Examiner's view, persons of ordinary skill in this art who read Ogawa would *not* be motivated to modify Ogawa with additional steps, procedures, or materials that were available in the art as Ogawa's method relies on the use of an ultrafilter (ultrafiltration membrane) to the exclusion of conventional methods.

Applicants submit that persons of ordinary skill in the art who read Ogawa would readily understand that ultrafilters fractionate all types of molecules (by retention or exclusion) solely based on their size, *not* on a binding to the filter surface. Thus, according to Ogawa's own teachings, it is necessary to select an ultrafilter having the specific size exclusion/retention properties that permit retention of molecules of the size of the desired phage DNA in order to recover such DNA, e.g., by adding TE buffer and shaking the membrane to solubilize the retained molecules (see, e.g., in Ogawa, col. 3, lines 31-38; col. 4, lines 23- 37). However, in Applicants' process, nucleic acids are ***not*** isolated because they are sufficiently large to be physically retained by an ultrafilter as employed by Ogawa. On the contrary, the size of the nucleic acids is irrelevant as nucleic acids are ***bound*** to one side of a non-siliceous membrane in a step that requires an immobilization buffer (also referred to as a "binding solution"), *not* by simply trapping or retaining the nucleic acids from passing through the membrane due to their sufficiently large size (see, below).

The difference between Ogawa and Applicants' invention is evident from the types of reagents that may be used in the two processes. For example, nucleic acids bound to a non-siliceous membrane according to Applicants' invention are readily released and recovered from the membrane using an *elution agent*, such as water or an aqueous solution having a relatively

low salt concentration. Prior to release, the bound nucleic acids may be washed. Washing buffers used in the invention may be the same as the binding solutions, whereas the elution agents *cannot* be the same as the binding solutions because binding solutions prevent release from the membrane and thus will decrease the yield of nucleic acids that can be recovered. See, e.g., the discussion of washing buffers and elution agents at p. 9, line 23-p. 10, line 10; Example 14 (a study of washing buffers); Example 15 (a study of elution buffers), of the specification. In contrast, Ogawa specifically teaches the use of the same buffer, such as TE buffer (a well known low salt buffer for solubilizing nucleic acids), to *wash and retrieve* the high molecular weight phage DNA that is retained on the ultrafilter:

> "The obtained filtrate was subjected to ultrafiltration through a[n] *ultrafilter having a fractionation molecular weight of 300,000* to remove low molecular weight components in the culture medium. To decompose the proteins in the phage, 5 μg/μl solution of proteinase K was placed on the ultrafilter, and the reaction was allowed to occur for 10 minutes.
>
> "The enzyme solution was removed under pressure, *and the ultrafilter was washed with TE buffer* by passing through the buffer under pressure through the ultrafilter. *Two hundred microliters of TE buffer* was placed on the ultrafilter and the ultrafiltration was shaken. Thereafter, the TE buffer was recovered by using a pipette." (col. 4, lines 23-37, of Ogawa; emphasis added)

Clearly, Ogawa's method involves an innately different process based on size fractionation that uses different materials and solutions than those used in Applicants' claimed process.

In addition, the difference between the binding chemistry employed in Applicants' claimed process and the size exclusion/retention ultrafiltration process employed in Ogawa (as well as the ultrafiltration prior art generally) is also reflected in the fact that the two procedures isolate different types of nucleic acids. As noted above, the ultrafiltration method of Ogawa can only isolate phage DNAs that have a sufficiently high molecular weight to be physically prevented from passing through the ultrafilter. A person of ordinary skill in this art understands that a requirement to use ultrafiltration *necessarily* requires that the person know the size of the molecule of interest that is to be retained or excluded from the ultrafilter and then select an ultrafilter having the particular size exclusion parameters necessary to retain or exclude the

molecule of interest. In contrast, in Applicants' claimed process nucleic acids are bound to a non-siliceous membrane so that there is *no restriction* on the particular size, configuration, type, or source of the nucleic acids that can be isolated (see, e.g., in Applicants' specification, p. 6, lines 16-22; Example 3 (isolation of total RNA from HeLa cells); Example 4 (isolation of RNA from an aqueous solution); Example 6 (isolation of genomic DNA)).

Applicants also note that the Examiner has mischaracterized Ogawa's own teachings. As noted in the above quote from the Office Action, the Examiner stated that the membrane in Ogawa can be *any* commercially available membrane (e.g., polysulfone) (p. 6, of the Office Action). In fact, Ogawa states that "the ***ultrafilter*** may be ***made*** of any material", such as, "commercially available ***ultrafilters*** made of polysulfone (see, col. 3, lines 38-39, of Ogawa; emphasis added), ***not*** that the ultrafilter may be any commercially available membrane. Accordingly, Ogawa does not teach or suggest substituting the ultrafiltration membrane with any membrane available in the art.

Applicants respectfully submit that the above comments clearly show that the process of Ogawa does not teach or suggest Applicants' claimed process. Persons of ordinary skill in this art who read Ogawa learn of a method of isolating a particular size of phage DNA using the size exclusion/retention process of ultrafiltration and to carry out such ultrafiltration to the exclusion of other conventional steps, methods, and materials. Nowhere does Ogawa teach, suggest, or provide motivation to persons of ordinary skill in the art to modify Ogawa by replacing the size exclusion/retention process of ultrafiltration with components in the art to make Applicants' method that involves binding nucleic acids to one side of a non-siliceous membrane under one condition (immobilization buffer) and releasing and recovering the bound nucleic acids under a different condition (elution agent).

In view of the above comments, Applicants respectfully submit that Ogawa neither teaches nor suggests Applicants' claimed process for isolating nucleic acids and, therefore, does not render Applicants' claims *prima facie* obvious.

As indicated in the above excerpt from the Office Action, the Examiner further relies on Pfister as a disclosure of the RNeasy® method for various protocols for isolating RNA and, in particular, the use of salt and alcohol to so modify Ogawa in order to make Applicants' claimed process for isolating nucleic acids. As shown above, Ogawa provides no motivation and actually

teaches against the use of conventional steps, such as alcohol precipitation, to isolate phage DNA. However, even if combined with Ogawa, the result does not provide the art with Applicants' claimed process. As explained below, just as the method of Ogawa is specifically directed to using ultrafiltration to isolate a particular size of phage DNA to the exclusion of other (smaller and larger) species, the RNeasy® method is designed with exacting requirements to isolate only a certain size population of RNAs to the exclusion of other species. A clear description of the RNeasy® method is provided in the RNeasy® Mini Handbook (05/99, attached as Exhibit A), an excerpt of which is reproduced for convenience here:

> **"The RNeasy Principle and Procedure**
> "The RNeasy procedure represents a novel technology *for RNA isolation.* This technology combines *the selective binding properties of a silica-gel-based membrane with the speed of microspin technology. A specialized high-salt buffer system allows up to 100 µg of RNA longer than 200 bases to bind to RNeasy silica-gel membrane.* Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine isothiocyanate (GITC)-containing buffer which immediately inactivates RNases to ensure isolation of intact RNA. *Ethanol is added to provide appropriate binding conditions and the sample is then applied to an RNeasy mini spin column where the total RNA binds to the membrane* and contaminants are efficiently washed away. High-quality RNA is then eluted in 30 µl, or more, of water.
>
> "The RNeasy Mini and RNeasy Plant Mini procedures isolate all RNA molecules *longer than 200 nucleotides. Small RNAs such as 5.8S RNA, 5S RNA, and tRNAs, approximately 160, 120, and 70-90 nucleotides in length respectively, will not bind quantitatively under the conditions used. Since low-molecular-weight RNA species make up 15-20% of the total RNA, the RNeasy procedure enriches for larger RNA molecules.* Therefore, the size distribution of RNA isolated with the RNeasy procedure is comparable to that obtained by centrifugation through a CsCl cushion, because small RNAs do not sediment efficiently during centrifugation through CsCl." (pp. 7-8, RNeasy® MiniHandbook, May 1999; bold in original, italics added for emphasis)

The above excerpt clearly shows that the RNeasy® method expressly and specifically *requires* "the selective binding properties of a silica-gel-based membrane" combined with "the speed available from microspin technology" and uses "a specialized high-salt buffer system" for

the sole purpose of binding and isolating RNA "longer than 200 nucleotides" (see, also, Table 1, p. 12, in Exhibit A). Thus, alcohol is added in the RNeasy® process to create conditions for the selective binding of the desired RNA population to the RNeasy® silica-gel-based membrane (see, e.g., p. 10, "Enzymatic Lysis Protocol" and "Mechanical Disruption Protocol", of the RNeasy® Mini Handbook, Exhibit A). Moreover, when the population of RNA molecules greater than 200 nucleotides is subsequently eluted from the silica-gel-based membrane, the released RNA is collected in the bottom of a microspin tube along with the TE buffer that has passed through to *the **other** side of the silica-gel-based membrane* (see, diagram, p. 9, of the RNeasy® Mini Handbook, Exhibit A). In contrast, in Applicants' claimed process, any and all species of nucleic acids are bound to one side of a *non-siliceous* membrane in the presence of an immobilization buffer and subsequently released and recovered from the membrane with an elution agent *wherein the released nucleic acids do not pass through to the other side of the non-siliceous membrane* and *wherein the released nucleic acids are removed without retrieving materials that have contacted the other side of the membrane*. Thus, the RNeasy® process involves conditions that are specifically excluded from those of Applicants' claimed process. Accordingly, Applicants respectfully submit there is no reasonable way a person of ordinary skill in this art could arrive at Applicants' claimed process by combining the diverse teachings of Ogawa and RNeasy®.

The Examiner also relies on Boom to provide additional components to the combination of Ogawa and RNeasy®:

> "For example, Boom teaches methods of purifying nucleic acids using various buffers (columns 6-7). Further Boom teaches that the methods are suitable for various nucleic acids (e.g. RNA, dsDNA, ssDNA). (col. 8, ll. 15). However, most importantly, the reference teaches that various surfaces, including siliceous and non-siliceous (e.g., silica derivatives, latex, PVDF, nitrocellulose, Hybond-N), can be utilized in purifying nucleic acids. (col. 6, ll. 5-27). Thus the evidence in the art suggests that optimizing buffers and immobilizing surfaces constitutes nothing more than routine experimentation. As a result, Applicant's implicit assertion that employing a step of immobilizing nucleic acids to a non-siliceous surface *in the presence of a salt and an alcohol*, cannot be deemed nonobvious." (pp. 7-8, of the Office Action; emphasis in original).

First, with respect to the Examiner's comment regarding Applicants' "implicit assertion", Applicants rely on the claims, as amended above, as the statement of their invention, which involves binding nucleic acids to one side of a non-siliceous membrane in the presence of a immobilization buffer (binding solution) and a subsequent release of the bound nucleic acids from the same side of the membrane in the presence of an elution agent wherein the released nucleic acids do not pass through to the other side of the membrane. As explained below, Boom cannot cure the deficiencies of Ogawa and RNeasy® to provide Applicants' claimed invention.

Boom describes the binding of nucleic acids in the presence of a chaotropic agent to various surfaces, such as silica coarse (SC) particles and also non-siliceous surfaces. However, there is an important difference between the methods of Boom that employ SC particles and those that employ non-siliceous surfaces. Boom describes various protocols for binding nucleic acids to SC and other silica particles in the presence of various chaotropic agents with subsequent elution of the nucleic acids from the particles using TE buffer (see, e.g., Protocol B at col. 7, line 60-col. 8, line 11; Protocol Y at col. 8, line 13-35; Protocol Y* at col. 8, lines 37-59; and Examples at col. 12, line 29-col. 20, line 49, of Boom). However, in the case of non-siliceous filters, nucleic acids are bound so tightly using the methods of Boom that elution of the bound nucleic acids with TE buffer *cannot* occur. Accordingly, Boom teaches a method of working not with the free nucleic acids, but with the filters containing the immobilized DNA:

> "Although normally no release of DNA takes place in the low salt buffer (Tris 10 mM-EDTA 1mM pH 8.0) this optional problem is set aside by inserting *the filter with DNA bound to it* in the PCR-reaction mix *instead of eluting the DNA from the filter*." (col. 22, lines 8-10, of Boom; emphasis added)

See, also, the description of Protocol Y** (col. 8, line 60-col. 9, line 13, of Boom) and Example I1 (col. 22, lines 16-64, of Boom). Clearly, Boom teaches that methods using SC and other silica materials are not simply interchangeable with various non-siliceous filters. Moreover, it is clear that no amount of routine optimization will remedy the problem of how to elute and retrieve nucleic acids bound to non-siliceous filters by the method of Boom. In fact, Applicants submit that their claimed invention actually provides a solution to the problem described in Boom of having to work with filters containing immobilized DNA by providing a process in which nucleic acids are bound to a non-siliceous membrane under one condition (in the presence of a

binding solution) and readily released from a non-siliceous surface under another condition (in the presence of an elution agent). Accordingly, Boom clearly cannot cure the deficiencies of Ogawa and RNeasy® but only adds problems that are solved by Applicants' invention.

Applicants respectfully submit that the above explanations clearly show that the combination of Ogawa, RNeasy®, and Boom neither teaches nor suggests Applicants' claims as amended herein. Accordingly, in view of the fact that the combination does not render Applicants' claims *prima facie* obvious under 35 USC 103, reconsideration and withdrawal of the rejections are respectfully solicited.

In view of all of the above comments, Applicants respectfully submit that the rejections have been rendered moot or overcome. Accordingly, the Examiner is respectfully requested to withdraw the rejections and pass the claims, as amended herein, to allowance.

Respectfully submitted,

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